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ABSTRACT

The use of a tilt platform to simulate a lateral ankle sprain and record muscle reaction time is a well-established procedure. However, a potential caveat is that repetitive ankle perturbation may cause a natural attenuation of the reflex latency and amplitude. This is an important area to investigate as many researchers examine the effect of an intervention on muscle reaction time. Muscle reaction time, peak and average amplitude of the peroneus longus and tibialis anterior in response to a simulated lateral ankle sprain (combined inversion and plantarflexion movement) were calculated in twenty-two physically active participants. The 40 perturbations were divided into 4 even groups of 10 dominant limb perturbations. Within-participants repeated measures analysis of variance (ANOVA) tests were conducted to assess the effect of habituation over time for each variable. There was a significant reduction in the peroneus longus average amplitude between the aggregated first and last 10 consecutive ankle perturbations ($F_{2.15,45.09}$ = 3.90, P = 0.03, η_p^2 = 0.16). Authors should implement no more than a maximum of 30 consecutive ankle perturbations (inclusive of practice perturbations) in future protocols simulating a lateral ankle sprain in an effort to avoid significant attenuation of muscle activity.

INTRODUCTION

The ankle sprain is the most prevalent injury amongst the sporting population (Junge et al., 2009; Swenson et al., 2013). It is reported that 77-83% of all ankle sprains are focal to the lateral ligament complex (Hawkins et al., 2001; Price et al., 2004; Woods et al., 2003), with excessive loading during inversion and plantar flexion the most common mechanism of injury (Ferran & Maffulli, 2006; Kaminski et al., 2013). When the foot is forced into a compromised position, the mechanoreceptors in the ankle ligaments and joint capsule sense the extreme or sudden movements and initiate a dynamic restraint (Hertel, 2002; Konradsen et al., 1997; Hertel, 2000; Jackson et al., 2009; Michelson & Hutchins, 1995). Peroneus longus activation provides a dynamic restraint against excessive inversion (Cordova & Ingersoll, 2003; Konradsen et al., 1997), whereas the tibialis anterior restrains excessive plantar flexion (Denyer et al., 2013; Mitchell et al., 2008; Vaes et al., 2002). Coordinated and correctly timed contraction of these muscles is vital in order to protect the ankle joint from injury (Berg et al., 2007), and is frequently referred to as a dynamic defence mechanism (Hertel, 2002; Konradsen et al., 1997; Thain et al., 2015).

The dynamic defence mechanism is typically evaluated by simulating a lateral ankle sprain (Eechaute et al., 2009; Silva et al., 2006; Wilson & Madigan, 2007), whereby the involved ankle undergoes a sudden perturbation into an inverted and/ or plantar flexed position, with the reaction time of the peroneus longus measured (Denyer et al., 2013; Konradsen et al., 1997; Thain et al., 2015). Studies have examined a variety of interventions aimed to improve the reaction time (faster) of the peroneus longus in response to the ankle perturbation in an effort to decrease the likelihood of injury (Cordova et al., 2010; Han & Ricard, 2011; Henry et al., 2010; Ramanathan et al., 2011).

Notwithstanding this, there is limited consideration of an attenuation of the reflex latency as a result of the repetitive ankle perturbations performed during testing. Consecutive ankle perturbations of three (Berg et al., 2007; Thain et al., 2015), five (Cordova et al., 2010; Henry et al., 2010), six (Hopkins et al., 2006; Hopkins et al., 2007; Palmieri-Smith et al., 2009; Mitchell et al., 2008; Vaes et al., 2002), 10 (Han & Ricard, 2011; Lynch et al., 1996) and 40 (Lynch et al., 1996) have been reported in the literature. The number of practice tilts is also selected heuristically, with some studies incorporating one practice trial before the main testing commences (Fernandes et al., 2000; Henry et al., 2010), others reporting 'several' trials (Berg et al., 2007; Hopkins et al., 2007), and others have no mention of practice at all (Ebig et al., 1997; Han & Ricard, 2011; Konradsen et al., 1997; Lynch et al., 1996; Mitchell et al., 2008; Palmieri-Smith et al., 2009).

Habituation can be defined as a decrease in the strength of a natural behaviour that occurs following repeated exposure to a stimulus (Bouton, 2007). The nervous system constantly evaluates incoming stimuli and only responds to those that are important (Rankin et al, 2009). In the instance of repeated ankle perturbations, it is possible that the reflex action of the dynamic defence mechanism becomes slower as the nervous system recognises that the stimulus is non-noxious (Keshmer et al., 1987). The habituation phenomenon has been studied in postural control in response to sudden perturbation of a supporting platform, whereby attenuation in the electromyography (EMG) reflex response with repetitive perturbations occurs in tibialis anterior, gastrocnemius and soleus (Hansen et al., 1988; Keshmer et al., 1987). Amplitude attenuation has also been identified in the upper extremity (Floeter et al., 1998), as seen in the cutaneomuscular reflex recorded from the dorsal interosseous muscle (Harrison et al., 2000). Additionally, the latency reflex of flexor digitorum superficialis has been shown to reach a state of habituation following 10 trials of a reaction task (Günendi et al., 2005).

Many studies investigate the effects of an intervention on neuromuscular control and use muscle reaction time as the outcome measure (Denyer et al., 2013; Thain et al., 2015). However, if habituation occurs, any change in reaction time post intervention could potentially be attributed to a naturally occurring habituation of reflex latency rather than the intervention itself. The reflex response of the peroneal muscles has been evaluated after landing from a jump onto an inverted surface, with no habituation occurring after 20 trials (Grüneberg et al., 2003). In contrast, when evaluated in the static position prior to the initiation of an inverted ankle perturbation, peroneus longus muscle activity significantly decreased, whilst the reaction time improved over time (Jackson et al., 2009), however, only 10 consecutive perturbations were performed. With conflicting findings and limited research, there is a need for further evaluation in this area. It is also important to determine if habituation occurs across other muscle groups contributing to dynamic ankle stability.

The aim of this study was to examine the effect of repetitive ankle perturbations on the reaction time, peak amplitude and average amplitude of the peroneus longus and tibialis anterior muscle groups.

METHODS

Participants

Twenty-two physically active participants (age = 21.1 ± 1.7 years, height = 1.7 ± 0.10 m, mass = 66.9 ± 8.1 kg) volunteered for the study after human participant approval was granted by the Institutional Ethics Committee. To meet the inclusion criteria, participants had to be aged 18 to 25 years; participate in sport at least once a week; have pain-free palpation of the anterior talofibular and calcaneofibular ligament; and have pain-free active dorsiflexion, plantar flexion, inversion and eversion range-of-motion. Exclusion criteria included: a previous ankle sprain; any current injury that would affect lower limb biomechanics, including acute trauma and muscular pain; use of foot orthotics; biomechanical abnormalities; and a history of lower limb surgery. Informed written consent was obtained in accordance with institutional guidelines.

Instrumentation

During all testing conditions, EMG data were collected using a Biometrics DataLog Wireless EMG system (model W4X8; Biometrics Ltd, Gwent, United Kingdom). All EMG signals were amplified and sampled at 1000Hz. The Biometrics DataLog system incorporated both a low pass filter (450Hz) and a high-pass third order filter (18dB/octave). The latter was designed to remove direct current offsets due to membrane potentials and to minimize low frequency interference caused by movement of the pre-amplifier on the skin surface. The pre-amplifier also contained an eighth order elliptical filter (-60dB at 550Hz).

A tilt platform was utilised that simulated a lateral ankle sprain (Figure 1). It consisted of two independent foot plates, so either foot could undergo sudden perturbation to reduce any anticipatory effect. The sprain simulation differed from previous platforms as 20° of plantar flexion was incorporated alongside 30° of inversion. The platform was constructed and first implemented by Mitchell et al. (2008). When the tilt was initiated, the contact switches would separate, thus sending a digital signal to the Biometrics DataLog system. The signal response time was 1 ms and was recorded on the display screen with an event marker.

Participant Preparation

Electromyographic data were recorded from the dominant lower limb, which was defined as the leg the participant would choose to stand on to maintain single leg balance. The peroneus longus and tibialis anterior muscles were actively contracted and palpated. The area was shaved with disposable razors and shaving foam, before being cleansed with isopropyl alcohol wipes to reduce skin impedance. Biometrics SX230 pre-amplified surface electrodes (gain x 1000; input impedance >1015Ω; common mode rejection ratio >96 dB; noise <5μV; bandwidth 20-60Hz) with a fixed 20 mm inter-electrode distance were affixed parallel to the orientation of the muscle fibres; peroneus longus – 1/4 on the line between the tip of the head of the fibula to the tip of the lateral malleolus; and tibialis anterior – 1/3 on the line between the tip of the fibula and the tip of the medial malleolus (Hermens et al., 2000). A ground reference cable (R206) was secured over the pisiform bone of the left wrist. Resisted ankle dorsiflexion, plantar flexion, inversion and eversion were performed to identify the response of the corresponding muscles with the EMG traces on screen.

Experimental Protocol

After the participant preparation took place, participants stood bare foot on the tilt platform and were instructed to disperse their weight evenly between both feet, whilst looking straight ahead in a relaxed stance. Participants stood shoulder width apart, feet parallel, and the two foot supports were adjusted accordingly to provide maximal support and safety. The researcher stood behind the participant to ensure the participant could not see the initiation of the tilt. To reduce the anticipatory effect either the dominant or non-dominant limb could undergo sudden perturbation. Additionally, the participant was engaged in conversation with the experimenter to distract their attention. The tilt was not initiated until the EMG signal trace was visually at a flat baseline. After the perturbation, the recording was stopped, the tilt platform was reset, and the remaining perturbations were carried out at 30 second intervals.

It was important to control the timing of the perturbations due to the nature of the study and the aim of identifying a possible habituation that may be time dependant. In order to reduce the anticipatory effect by the participant counting the 30 second intervals, participants were engaged in conversation with the experimenter to distract their attention. This procedure was performed until 40 perturbations had been recorded for the dominant limb. Forty perturbations were selected to replicate the maximal number of consecutive ankle perturbations performed within the literature (Lynch et al., 1996).

Data Processing

The 40 perturbations were divided into 4 even groups of 10 dominant limb perturbations (Group 1: trial 1 to 10; Group 2: trial 11 to 20; Group 3: trial 21 to 30; Group 4: trial 31 to 40). It was therefore important to control for the duration between the first and 10th dominant limb perturbation within each group. A random sequence was computed via the use of a random sequence generator (Excel 2010), that ensured there were 5 non-dominant and 10 dominant limb perturbations within each group totalling 15 perturbations (Table 1). The sequence was controlled so that the first and last perturbation in each group was always a dominant limb perturbation. The testing duration from the first to 10th dominant limb perturbation was controlled at 7 minutes. No practice perturbations were administered to ensure any possible attenuation of peroneus longus and tibialis anterior reflex response was fully evaluated.

Raw EMG signals were visually checked for artefacts and then processed using a root mean squared (RMS) filter with a moving window length of 10 ms (Biometrics Ltd, DataLog Software, Version 7.5). The filtered signal was exported into a spreadsheet, before a specially designed computer onset detection method calculated the onset of EMG muscle activity, and then subsequent muscle reaction time, peak and average amplitude. To calculate the onset of EMG muscle activity, the EMG readings had to be elevated by five (peroneus longus) and ten (tibialis anterior) standard deviations above the 1000 ms baseline reference point obtained prior to the tilt, for a consecutive 20 ms period for the muscle to be deemed active. The different standard deviations used as a threshold in the computer onset detection algorithm are set in accordance with unpublished work by Thain (2013). In the work by Thain, the signal-to-noise ratio at baseline was different for the two muscles when standing on the tilt platform in a relaxed stance. Therefore to reduce the probability of performing a type I error, a higher threshold for tibialis anterior needed to be set. Muscle reaction time was calculated as the time (ms) between the initiation of the tilt and the onset of muscle activity. The peak and average amplitude were calculated within a 100 ms window starting from when the muscle was deemed active.

Statistical Analysis

All statistical tests were performed on IBM SPSS Statistics Version 20 (IBM Corporation, Armonk, NY). The 40 consecutive measurements for each participant were divided into four groups of 10 trials each (Group 1: trial 1 to 10; Group 2: trial 11 to 20; Group 3: trial 21 to 30; Group 4: trial 31 to 40). After the mean was calculated for each group, normality of the data was verified (Shapiro-Wilk, P > 0.05). Within-participants repeated measures analysis of variance (ANOVA) tests were conducted to assess the effect of habituation over time for each variable. In cases when the assumption of sphericity had been violated according to Mauchly's test ($P < 0.05$); the Greenhouse-Geisser procedural adjustment for degrees of freedom was applied. When the ANOVA provided a significant main effect, post hoc pair wise comparisons between each of the groups were performed using the Bonferroni adjustment for multiple comparisons. The alpha level of significance was set at $P < 0.05$. Effect sizes (η_p^2 values) were also calculated (0.01 = small; 0.06 = medium; 0.14 = large) (Pallant, 2010).

RESULTS

The average amplitude demonstrated a significant main effect of habituation for peroneus longus ($F_{2.15,45.09} = 3.90$, $P = 0.03$, $\eta_p^2 = 0.16$) (Figure 2). Post hoc tests identified a significant difference in average amplitude between the first and last group of consecutive ankle perturbations only ($P = 0.03$). There was no significant main effect for habituation in tibialis anterior average amplitude ($F_{1.61,33.89} = 2.17$, $P = 0.14$, $\eta_p^2 = 0.09$). There was no significant main effect of habituation for peak amplitude of peroneus longus ($F_{1.85,38.88} = 1.62$, $P = 0.21$, $\eta_p^2 = 0.07$) or tibialis anterior ($F_{1.90,39.91} = 1.36$, $P = 0.27$, $\eta_p^2 = 0.06$) (Figure 3). Likewise, there was no significant main effect of habituation for peroneus longus ($F_{2.12,44.54} = 0.75$, $P = 0.49$, $\eta_p^2 = 0.03$) or tibialis anterior ($F_{1.51,31.74} = 1.03$, $P = 0.35$, $\eta_p^2 = 0.05$) reaction time to repetitive ankle perturbations (Figure 4).

DISCUSSION

The purpose of this study was to examine a possible habituation of muscle reaction time, peak amplitude, and average amplitude in response to repeated ankle perturbations. The findings show that there is an attenuation of peroneus longus average amplitude with repetitive ankle perturbations, but no change in peak amplitude or reaction time.

There was a significant decrease (*P* = 0.03) in the average amplitude of peroneus longus in response to a simulated ankle sprain (Figure 2), with a large effect size (η_p^2 = 0.16). Post hoc tests revealed that the difference was between the first group of 10 consecutive perturbations and the final group of perturbations. Attenuation of the amplitude has previously been observed by others (Bloem et al., 1998; Hansen et al., 1988; Jackson et al., 2009; Keshmer et al., 1987). This has been attributed to the body familiarising itself with the mechanism imposed on the ankle and quickly learning that the stimulus is non-noxious and thus conserves energy and motor recruitment (Keshmer et al., 1987). This may explain the progressive attenuation over time, although one would have expected similar findings in tibialis anterior.

Specifically to the ankle, attenuation of the muscle activity has been shown to occur throughout 10 consecutive ankle inversion perturbations, with a more drastic decline in activity of tibialis anterior in comparison to the peroneus longus or brevis (Jackson et al, 2009). The mechanism of simulating a lateral ankle sprain by Jackson et al. (2009) was void of plantar flexion. Therefore the increased decline of the tibialis anterior muscle activity may have occurred because of a reduced stretch on the tendons, and as a result, the body realising it was not required in response to an inversion only perturbation. However, in the current study, the simulation of a lateral ankle sprain was inclusive of plantar flexion and therefore tibialis anterior was required to consistently react throughout trials. Consequently, rather than attributing the findings of a decline in peroneus longus average amplitude to the muscle familiarising itself with the mechanism imposed on the ankle - as the same was not seen for tibialis anterior - a preferred rationale is that the peroneus longus became fatigued.

Previous research has outlined that the attenuation of muscle amplitude is exacerbated by fatigue in peroneus longus (Jackson et al. 2009). However, the methods in the current study are novel, and as such, comparison of results with other studies should be treated with caution. Jackson et al. (2009) used a seated isokinetic inversion fatigue protocol with an eccentric focus, which is highly controlled and repeatable, but the mechanism of an ankle sprain is far removed from this mechanism of fatigue. The normal kinetic chain of the human body was not fatigued, but rather localised fatigue of one segment in the kinetic chain. Jackson et al. (2009) identified an attenuation of the amplitude after 10 consecutive perturbations, yet in the current study, a significant attenuation was not elicited until 31-40 ankle perturbations had been performed. As the current study stressed the entire kinetic chain in normal bi-pedal weight bearing stance, the neuromuscular impact of the consecutive perturbations and any fatiguing as a result, was felt by the whole kinetic chain, both on the tilt limb and the support limb. Therefore it is plausible that without a focused fatiguing protocol, it took longer for the attenuation to present. This is supported with a moderate effect of habituation for peak amplitude of peroneus longus ($\eta_p^2 = 0.07$).

It is only possible to speculate why the tibialis anterior did not respond in the same way, but potentially the tibialis anterior is more fatigue resistant or the peroneus longus may be the main muscle contributing to the dynamic defence mechanism. When Koradsen et al. (1997) first coined the 'dynamic defence mechanism' they referred solely to the evertors, the peroneus muscles, and performed an inversion only ankle perturbation. It was subsequent researchers that decided to implement a plantar flexion component into the ankle perturbation to better simulate a lateral ankle sprain mechanism of injury (Mitchell et al., 2008; Vaes et al., 2002). It was assumed that tibialis anterior may also contribute to the dynamic defence mechanism by resisting forced plantar flexion. However, the contribution of each muscle remains unknown, and therefore future research should compare peroneus longus and tibialis anterior muscle amplitude in response to an inversion and plantar flexion, and an inversion only simulated lateral ankle sprain.

The results of the current study show a reduction in peroneus longus average amplitude, but no significant difference in reaction time or peak amplitude. The participants experienced 40 ankle perturbations at approximately one perturbation every 30 seconds, with the focus of the dynamic defence mechanism being on the peroneus muscles. The authors believe that the 40 perturbation protocol may have elicited a fatiguing effect on the limb being tilted, that manifested itself as a distortion of the somatosensory afferents, resulting in a compromised sensorimotor system. This has been observed by Doherty et al. (2015) in individuals recovering from a lateral ankle sprain. This short term reduced neuromuscular control at the ankle, specifically focal to the peroneus muscles, requires a modification of the strategy used to maintain stability, which in this case is a move away from the ankle strategy to the hip strategy; this is often seen in individuals with functional ankle instability (Pinstaar et al., 1996).

The muscles at the hip are larger, stronger, more fatigue resistant, have shorter levers and short tendons compared to those at the ankle. Doherty et al. (2015) observed significant increases in hip muscle activity during drop landings in participants tested within two weeks of sustaining a lateral ankle sprain compared to healthy controls. They suggested that the body unloaded the lateral ankle by exhibiting a modified centrally mediated motor response to compensate for deficits within the kinetic chain. This is the manifestation of the cerebellum and motor control centre, which crave stability, adapting to the situation to provide stability. When the distal ankle joint is injured (Doherty et al., 2015) or in the current study, possibly fatigued, the human body off-loads the ankle and provides stability and control using the proximal hip joint musculature. In this way, the dynamic defence mechanism still occurs within a duration to still be effective (no change in peroneus longus reaction time or peak amplitude), but with a contribution to the overall response from the hip musculature (reduction in average peroneus longus amplitude).

The results from this study show that administering consecutive perturbations causes a significant attenuation of the peroneus longus average amplitude. Notwithstanding, this study recommends that authors should implement no more than a maximum of 30 consecutive ankle perturbations (inclusive of practice perturbations) in future protocols simulating a lateral ankle sprain in an effort to avoid significant attenuation of muscle activity. Furthermore, as there were no significant differences between the first group of 10 perturbations and the second for any variables, it is prudent to suggest that practice tilts may not need to be prescribed. Moreover, it could be argued that the first reaction time provides the most accurate representation of motor recruitment when exposed to a potential deleterious mechanism of injury; this requires further examination.

A limitation of this study is that each of the four groups contained 10 ankle perturbations. Therefore, it is unable to depict the exact point when the attenuation becomes significant. Additionally, during the testing procedure only the dominant limb was used, however, future work should examine if differences exist between limbs. Furthermore, in an effort to distract the participant from counting the 30s intervals between tilts, casual conversation occurred. However, in an effort to standardise procedures, perhaps a video asking random questions in order to keep the subject engaged and concentrating could be delivered.

Future research should examine the role of the support limb in the double limb stance to establish if a habituation effect is also present. Additionally, other muscles that may contribute to the dynamic defence mechanism such as gluteus medius, extensor digitorum longus and peroneus brevis should be examined. Hopkins et al. (2006) simulated a more dynamic injury mechanism with participants walking on a tilt platform prior to experiencing an inversion ankle perturbation. Researchers should fully examine the dynamic defence mechanism by initiating simultaneous inversion and plantar flexion of the ankle during walking. Also, research should examine the differences in muscle recruitment between an inversion only and inversion and plantar flexion ankle perturbation.

CONCLUSION

The current study identified that a there is an attenuation of peroneus longus muscle activity with repeated ankle perturbations. Authors should implement no more than a maximum of 30 consecutive ankle perturbations (inclusive of practice perturbations) in future protocols simulating a lateral ankle sprain in an effort to avoid significant attenuation of muscle activity.

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CAPTIONS TO TABLES

Table 1: Random Sequence of Ankle Perturbations

Tilt Number	Tilted Limb
1	D
2	ND
3	D
4	D
5	ND
6	ND
7	D
8	D
9	D
10	ND
11	D
12	D
13	ND
14	D
15	D

Note: D = Dominant; ND = Non Dominant

CAPTIONS TO FIGURES

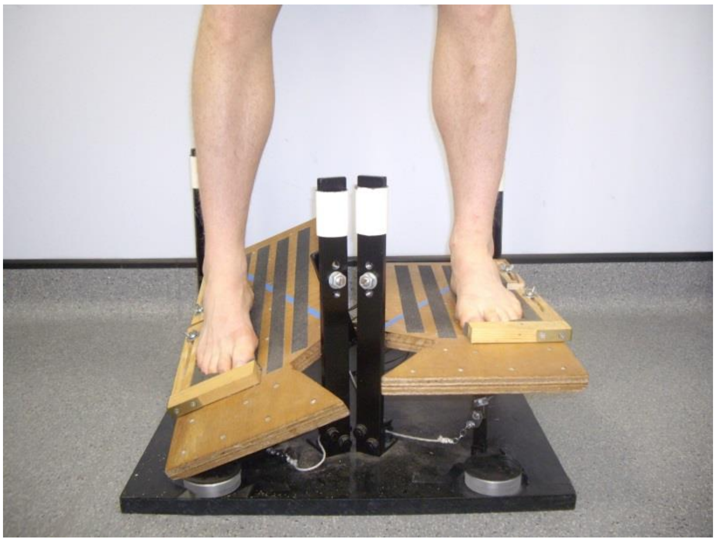

Figure 1: Tilt platform simulating a nonpathological lateral ankle sprain used for muscle reaction time testing.

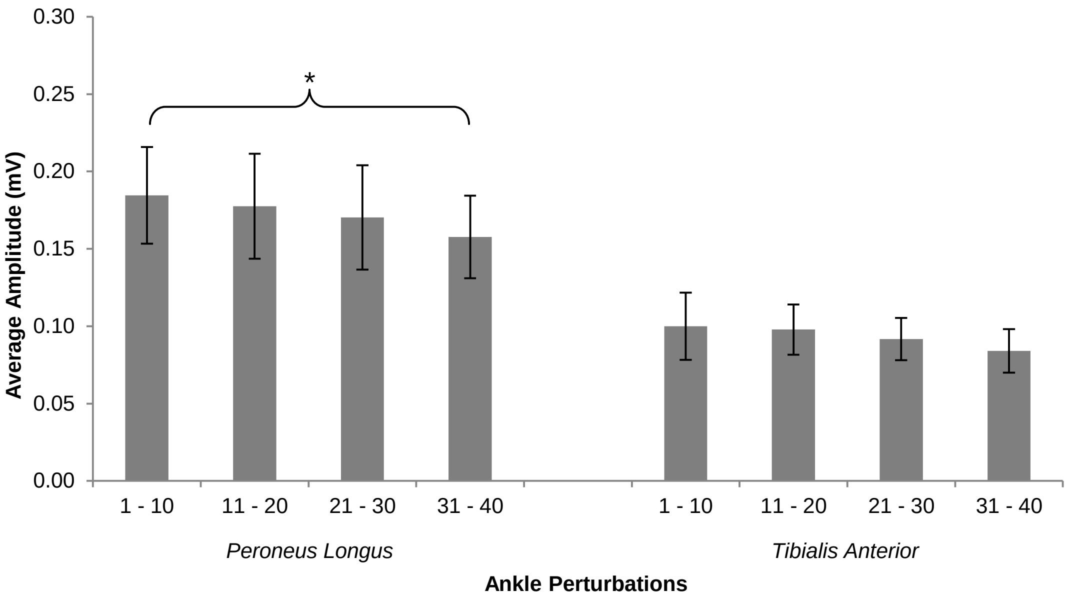

Figure 2 - The effect of repeated ankle perturbations on average amplitude (mean ± 95% Confidence Interval). (* P = < 0.05).

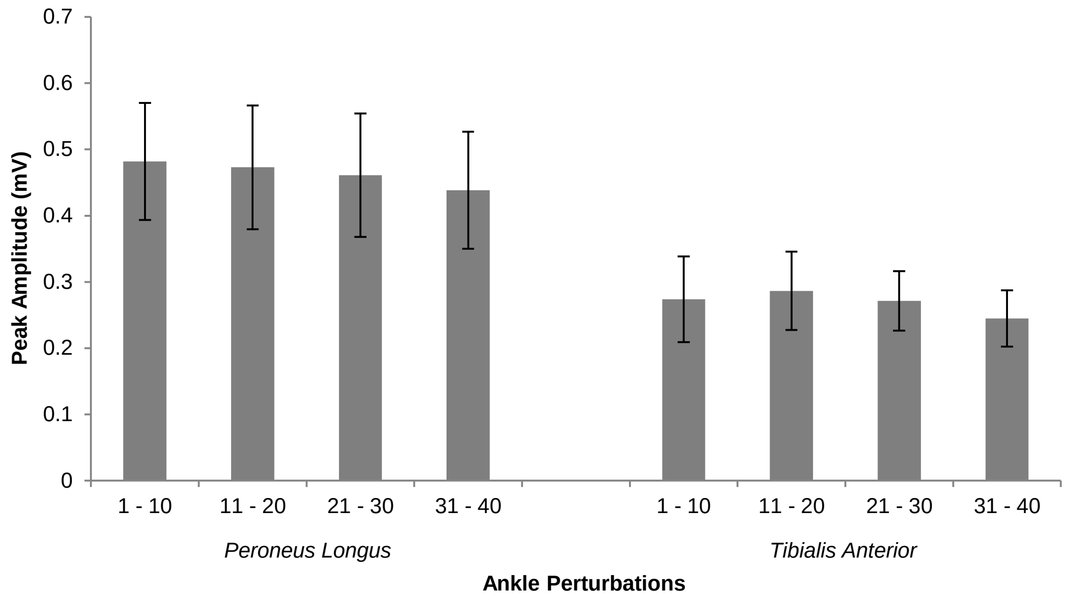

Figure 3 - The effect of repeated ankle perturbations on peak amplitude (mean ± 95% Confidence Interval).

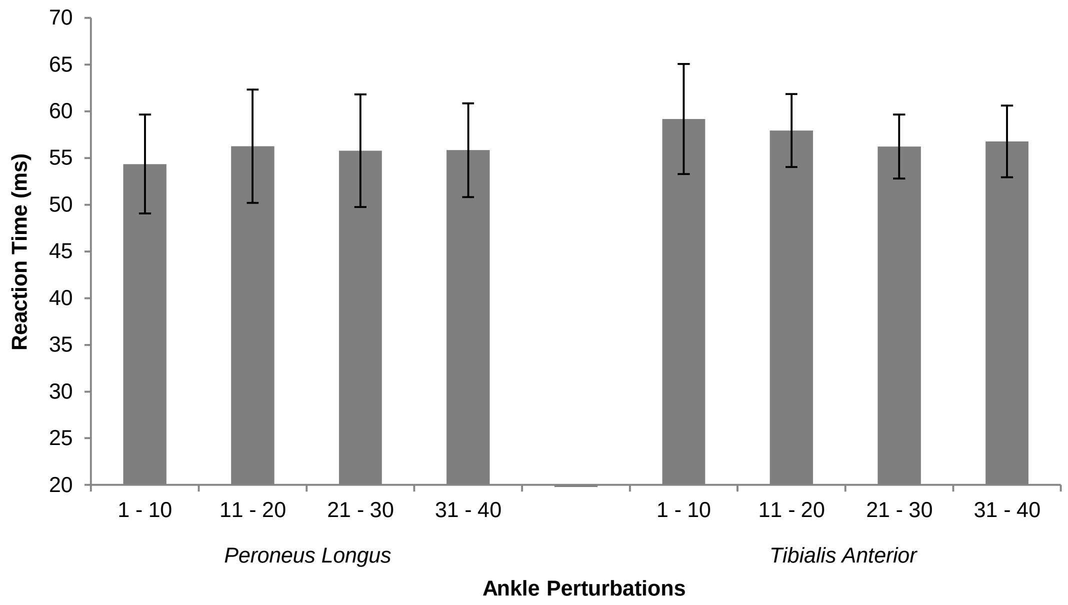

Figure 4 - The effect of repeated ankle perturbations on muscle reaction time (mean ± 95% Confidence Interval).